UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/582,534	06/27/2000	YVES POIRIER	MOBT:191/KAM	6807

7590 03/11/2003

Patrea L. Pabst
HOLLAND & KNIGHT LLP
One Atlantic Center
Suite 2000
Atlanta, GA 30309-3400

EXAMINER	
SCHMIDT, MARY M	
ART UNIT	**PAPER NUMBER**
1635	14

DATE MAILED: 03/11/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/582,534	POIRIER ET AL.	
	Examiner	Art Unit	
	Mary M. Schmidt	1635	

-- The MAILING DATE of this communication app ars on the cover sheet with the correspond nce addr ss --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 March 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *82-133* is/are pending in the application.

4a) Of the above claim(s) *133* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *82-86,91,92,97-104 and 109-132* is/are rejected.

7)☒ Claim(s) *87-90, 93-96 and 105-108* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *06-27-00* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Group III, claims 82-132 was elected in Paper No. 5, filed 12/26/00. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election was treated as an election without traverse (MPEP § 818.03(a)). The species elected were: claims 84, 113 and 124, *Saccharomyces cerevisiae*; claim 86, 92, 98 and 130, canola/oilseed rape.

2. Claim 133 stands withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in Paper No. 5.

Claim Rejections - 35 USC § 102

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

4. Claims 82, 85, 109, 112, 114, 121, 123, 125 and 128 are rejected under 35 U.S.C. 102(b) as being anticipated by Hahn et al. (International Symposium on Bacterial

Polydydroxyalkanoates '96, (abstract and poster), 16 pgs., 1996, "Peroxyisomal Localization of PHA Synthesis in Eukaryotic Cells").

Claim 82 is drawn to a recombinant host cell comprising a nucleic acid segment encoding a non-naturally occurring fusion protein, wherein the nucleic acid segment comprises:

a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit.

Hahn et al. taught maize plant and *A. eutrophus* bacteria transformed with peroxisomally targeted synthases. They show the plant transformation plasmids pACE 2px, 3px and 4px having a CaMV 35 S promoter and NoS terminator for expression of phbA, phbB and phBCpolyhrdroxyalkanoate synthase protein subunits and with the peroxisomal targeting subunit Arg-Ala-Val-Ala-Arg-Leu-COOH at the carboxy terminus. Note that the instant specification as filed teaches on page 2, lines 1-6, that polyhydroxyalkoates (PHAs) are a family of which the polyhydroxybutyrate (PHB) is the most well characterized. The PHA and PHB discussed in Hahn et al. are the same compounds.

Claim 109 is drawn to a method of preparing host cells useful to produce a non-naturally occurring fusion protein comprising the steps of:

(a) selecting a host cell;

(b) transforming the selected host cell with a recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

(I) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

(ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; and

c) obtaining transformed host cells.

Claim 114, states that the host cell is a plant.

Since Hahn et al. taught making plant vectors having a peroxisome targeting subunit and a nucleic acid sequence encoding three different PHB pathway genes, phbA-C, as well as insertion of synthase to produce PHB from D-3-HB-CoA, and transformation of the plants with the vector constructs, they taught making a plant host cell by the claimed method steps.

Claim 112 is anticipated by Hahn et al. since they taught the design of vectors for expressing a FOX3 gene animo-terminal peroxisomal targeting signal with a PHA synthase in yeast, a fungus, and the method step of inserting, transforming, the yeast to obtain the transformed cells.

Claim 121, drawn to methods for the preparation of a polyhydroxyalkanoate via making the cell having the peroxisome targeting protein subunit and polyhydroxyalkanoate synthase protein subunit and culturing the cell, is also anticipated by Hahn et al. as shown above. Similarly claim 128 is anticipated since the steps of obtaining a plant capable of producing a non-

naturally occurring fusion protein wherein the fusion protein comprises the peroxisome targeting protein subunit and the polyhydroxyalkanoate synthase protein subunit, and growing the plant under conditions suitable for the production of the polyhydroxyalkanoate, are all taught by Hahn et al. in their maize transformation example. They taught generation of elevated PHB levels over background levels.

Claim 123, which specifies that the host cell of claim 121 is a fungus, is anticipated by the teachings of Hahn et al. for practicing their methods in yeast. Claim 125, which specifies that the host cell of claim 121 is a plant, is anticipated by the teachings of Hahn et al. for practicing their methods in maize.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 82-86, 91-92, 97-104 and 109-132 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Hahn et al. (International Symposium on Bacterial Polydydroxyalkanoates '96, (abstract and poster), 16 pgs., 1996, "Peroxyisomal Localization of PHA Synthesis in Eukaryotic Cells") as applied to claims 82, 85, 109, 112, 114, 121, 123, 125 and 128 above, in view of

Bright et al. (WO92/19747, IDS Reference B5) and Hayashi et al. (IDS Reference C4; *The Plant Journal*, 10(2):225-234, 1996) and further in view of the combination of Elgersma et al. (JBC 271, 42, p. 26375-26382, 1997), Verleur et al. (Eur. J. Biochem. 247, 972-987,1997), Gengenbach et al. (U.S. Patent 6,146,867), Tomes et al. (U.S. Patent 6,258,999 B1), and Bright et al. (U.S. Patent 6,175,061 B1).

Hahn et al. is relied upon to teach peroxisomal localization of PHA synthesis in yeast and plants. He teaches the limitations of instant claims 82, 85, 109, 112, 114, 121, 123, 125 and 128 as set forth above. While he provides the vector construct for administration to yeast and teaches that "work is underway to insert a targeted PHA synthase into yeast", and the method steps, he does not further teach the isolated cell comprising the fusion construct (instant claims 83 and 84). He does not teach administration to canola/oilseed rape plant per se (instant claim 86). While he teaches administration of synthase, ketothiolase, and reductase, he does not specifically teach administration of an acyl-ACP thioesterase, a fatty acyl hydroxylase, yeast multifunctional protein (MFP0 or hydroxyacyl-CoA epimerase (instant claims 87-90, 93-96 and 105-108). He teaches yeast and plant vector constructs having a 5' promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, a fusion protein of a nucleic acid sequence encoding a peroxisome targeting protein subunit and a polyhydroxyalanoate synthase protein, a 3' transcription terminator sequence. He does not explicitly state that his vectors contain a 3'-polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of the transcribed RNA (instant claims 91-92,

97-98). He teaches use of the CaMV 35 S promoter (instant claims 99-100), but does not teach explicit use of the instantly claimed inducible and tissue specific promoters (instant claims 101-104). He taught the methods of claims 109, 112, 114, 121, 123, 125 and 128, but did not teach the selectable markers of claims 110-111, 117-118, the use of *Saccharomyces cerevisiae* (claim 113, 124), use of canola/oilseed rape (claim 115, 119, 126, 130), regeneration of the transformed host plant cell (claim 116, 120), wherein the culture contains fatty acids, non-natural fatty acids, or mixtures thereof (claim 122, 129), nor the generation of specific PHAs such as those claimed in instant claims 127, 131, 132.

Bright et al. is relied upon to teach production of polyhydroxyalkanoates in plants, such as the "oil-seed" crops including oilseed rape, canola, soya and sunflower (page 3, lines 24-31). Although they primarily teach targeting the plastid for production of the PHAs, they teach that using the same enzymes Hahn et al. taught, PHAs of different lenghts are produced (see figure 1, product). They further taught use of expression vectors for canola/oilseed rape having kanamycin as a selection gene (figure 3). They do not teach a fusion comprising a peroxisome targeting protein.

Hayashi et al. is relied upon to teach fusion proteins for transport to peroxisomes in plants. They do not teach fusion of polyhydroxyalkanoates.

Elgersma et al. and Verleur et al. are relied upon to teach peroxisomal targeting and signals to peroxisomes in *Saccharomyces*. They do not teach fusion of polyhydroxyalkanoates

for production in the peroxisome. Verleur et al. also teaches growth of *Saccharomyces* in specific nutrient, oleate, growth conditions.

Gengenbach et al. (U.S. Patent 6,146,867) is relied upon to teach use of constitutive plant promoters.

Tomes et al. (U.S. Patent 6,258,999 B1) is relied upon to teach inducible plant promoters.

Bright et al. (U.S. Patent 6,175,061 B1) is relied upon to teach tissue specific plant promoters.

It would have been *prima facie* obvious at the time the invention was made for one of ordinary skill in the art to make a recombinant host cell, a plant such as canola/oilseed rape or fungus such as *Saccharomyces cerevisiae*, having a fusion protein comprising a peroxisome targeting protein subunit and a nucleic acid encoding a polyhydroxyalkanoate synthase protein subunit, and wherein the nucleic acids are expressed from constitutive, inducible or tissue specific promoters (such as those taught in Gegenbach et al., Tomes et al. and Bright et al.), since: (1) Hahn et al. taught the delivery of fusion proteins having a peroxisome targeting signal for the production of PHAs and PHB in yeast and plants, (2) Hayashi et al., Elgersma et al. and Verleur et al. taught fusion proteins for transport to peroxisomes in plants and *Saccharomyces cerevisiae*, and it would have been *prima facie* obvious to substitute their targeting signals for those taught by Hahn et al., and (3) since Hahn et al. taught specifically making the

polyhydroxyalkanoates in the peroxisome, it would have been *prima facie* obvious to make the polyhydroxyalkanoates taught by Bright et al. in the peroxisome of a plant or yeast.

One of ordinary skill in the art would have been motivated to make a recombinant plant, such as canola/oilseed rape, or *Saccharomyces cerevisae* having a fusion protein comprising a peroxisome targeting protein subunit and a nucleic acid encoding a polyhydroxyalkanoate synthase protein subunit since (1) Hahn et al. taught motivation for synthesis of PHA via peroxisomal localization, (2) peroxisome targeting subunits were well-known in the art as exemplified by Hayahi et al. in plants and Elgersma et al. and Verleur et al. in *Saccharomyces cerevisae*; and (3) in addition to the PHA synthesis taught by Hahn et al., production of specific polyhydroxyalkanoates in plants was well known in the art as taught by Bright et al.

One of ordinary skill in the art would have had an expectation of success to localize nucleic acid sequences to the peroxisome via making a fusion of a peroxisomal signal and a nucleic acid expressing a polyhydroxyalkanoate since (1) peroxisomal targeting signals were well-known in the art at the time the invention was made as taught by Hayashi et al. , Elgersma et al. and Verleur et al., (2) synthesis of polyhydroxyalkanoates was well-known in the art at the time the invention was made as taught by Bright et al., and (3) Hahn taught success of localization of polyhydroxyalkanoate synthesis via peroxisome targeting.

Response to Arguments

7. Applicant's arguments filed 3/12/02 have been fully considered but they are not persuasive.

Applicant notes that a copy of the Hahn et al. reference was not supplied with the first Office action. The Hahn et al. reference has not become available and is supplied with the instant action, which is made non-final. Since applicant's arguments do not address the Hahn et al. reference, the arguments are not considered to overcome the rejection as it stands in view of the Hahn et al. reference now supplied.

Applicant has argued and discussed the Bright, Somerville et al., Elgersma et al., Hayashi and Verleur et al. references individually without clearly addressing the combined teachings with Hahn et al. It must be remembered that the references are relied upon in combination and are not meant to be considered separately as in a vacuum. It is the combination of all of the cited and relied upon references which make up the state of the art with regard to the claimed invention. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants arguments are that the Bright, Somerville et al., Elgersma et al., Hayashi, Verleur et al. references do not teach targeting to peroxisomes or that peroxisomes would contain appropriate substrate for PHA production. However, the reference of Hahn et al. is

provided to describe this motivation and expectation of success in the prior art in combination of the Bright, Somerville et al., Elgersma et al., Hayashi, Verleur et al., Hitz., Gengenback et al. And Tomes references.

Applicants state on pages 4 and 5 of the response that "None of the prior art, alone or in combination, teaches one to target enzymes involved in PHA production to the peroxisome, much less that one would have a reasonable expectation of success if one did so. Additionally, except in hindsight based on the applicant's disclosure, there is no suggestion to one of ordinary skill in the art to combine the cited references and derive applicant's claimed methods and compositions. The processing of substrate in the pathway leading to PHA synthesis is coordinately regulated. This coordinated activity relies upon the presence of substrate. The Applicants have shown that by increasing the specific substrates required for PHA synthesis, via fatty acid processing in the peroxisomal beta-oxidation cycle, increased PHA end product is observed when peroxisomally localized enzymatic activity is present. None of the cited prior art shows 1) that appropriate substrates for PHA synthesis exist in the peroxisome, 2) how one would obtain enough appropriate substrate to produce PHAs, or 3) how PHA synthesis is compartmentalized within a recombinant cell via the localization of substrate and the localization of coordinated enzymatic activity to the peroxisome. Again, the enzymes leading to PHA production have specific substrates to which they bind and catalyze their formation into a product that will be specifically utilized by the next gene encoded enzyme in the pathway. Enzymes dedicated to PHA synthesis are functioning in a specific and coordinated pathway therefore

resembling a multi-step processing unit. None of the prior art, alone or in combination, provides motivation to localize enzymes to the peroxisome, specifically for the purpose of PHA synthesis, because such localization would not provide for a PHA end product without the proper substrate feeding into the pathway. Without knowledge of the proper substrate present in the peroxisome, there would not be a reasonable expectation of success for the production of PHA. Furthermore, the localization of a single enzyme would only serve to process a single substrate (if it is present). As mentioned in the foregoing discussion, PHA synthesis relies upon a "chain" of enzymatic activities with each enzyme link in the chain processing the substrate produced from the previous enzymatic link. This coordinated enzymatic processing results in polymer. Accordingly, the prior art does not meet the legal standard under 35 USC 103 for making obvious the claimed subject matter."

In response, the Hahn et al. reference in the first sentence specifically states that "In an attempt to broaden the useful host range for PHA production and to improve the PHA yields achievable in eukaryotic cell systems, we are exploring the localization of PHA synthesis to the peroxisome." They further show in figure 1 the possible pathways for generation of PHA precursors through beta-oxidation in the peroxisome. Thus the Hahn et al. reference taught

As stated in the rejection filed 08/29/01, the Hahn et al. reference teaches the mechanism of PHA synthesis in peroxisomes and motivation for synthesis of PHA via peroxisomal localization. Hahn et al. teaches specific use of several different enzymes known in the PHA synthesis, beta-oxidation pathway. Thus although they only looked at overall PHA expression

levels, there was an expectation of success in the art that one of skill in the art would have been able to have made the different known polyhydroxyalkanoates such as those taught by Bright et al. since it was known that one of skill in the art would be able to synthesis PHAs in the peroxisome as taught by Hahn et al.

8. Claims 87-90, 93-96 and 105-108 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. The prior art did not teach nor fairly suggest specific design and administration of the acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein, MFP, or hydroxyacyl-CoA epimerase fusion proteins for delivery to the peroxisomes in the claimed constructs.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to *Mary M. Schmidt*, whose telephone number is (703) 308-4471.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, *John LeGuyader*, may be reached at (703) 308-0447.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group Analyst, *Katrina Turner*, whose telephone number is (703) 305-3413.

M. M. Schmidt
March 10, 2003

JOHN L. LeGUYADER
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600